2.1. Stock plant and seed production

Forty T. fasciculata var. fasciculata plants with immature in¯orescences were purchased from a commercial producer (Tropi¯ora, Sarasota, FL), who imported them from Honduras. The stock plants were grown in a glass greenhouse with a maximum PPF of 400 mmol mÿ2 sÿ1. The low and high average mean temperature was 238C and 32_28C, respectively. The average mean low and high humidity was 65 and 98%, respectively. At anthesis the plants were self-pollinated by hand. Each pollinated plant produced 15±30 capsules that took about a year to mature. Mature capsules were brown. They were harvested before they dehisced. Harvested capsules were cleaned and disinfested in 10% Clorox for 10 min, rinsed in tap water, air-dried and kept in open plastic containers to let them dehisce. Each capsule contained one to two hundred seeds.

2.4.1. Micropropagation of wild type T. fasciculata var. fasciculata

The trichomes of each seed were trimmed, then seeds were disinfested in 10% Clorox for 10 min, transferred to the laminar ¯ow hood where they were rinsed three times with sterile distilled water before being placed in test tubes containing basal media. Twenty seeds were used in each treatment (n.20). Two seeds were put in each 12 mm_94 mm test-tube with 13 ml of medium. The basal medium consisted of half strength MS salts and vitamins (Murashige and Skoog, 1962), 15 g lÿ1 sucrose, 8 g lÿ1 Difco±Bacto agar and a pH of 5.7. The treatments differed in their equimolar concentrations of BA and IBA, which were 0 (control), 0.01, 0.03, 0.1, 0.15, 0.20, 0.25, 0.30, 0.50, 1.0, 3.0 and 10.0 mM BA and IBA.

3.3.1. Micropropagation of wild type T. fasciculata var. fasciculata

Since T. fasciculata var. fasciculata seedlings are very slow growing, it was not necessary to transfer them to fresh media during the 6 months of the experiment. No multiplication occurred in the media with equimolar BA and IBA at 0, 0.01, 0.03, 0.1 and 0.15 mM (Table 2). Each seedling in the media with equimolar BA and IBA at 0.20 and 0.25 mM formed several shoots. Equimolar BA and IBA at 0.30 and 0.50 mM, respectively, produced 68% and 75% of seedlings with spherical clumps of shoots. One hundred percent of the seedlings in the media with equimolar BA and IBA at 1.0, 3.0 and 10.0 mM formed spherical clumps of shoots. The size of these clumps of shoots increased with the BA and IBA concentrations in the medium (Table 2). Two spherical clumps of shoots were removed from the media containing equimolar BA and IBA at 0.3, 0.5, 1.0, 3.0, and 10.0 mM. Each clump of shoots was halved and cultured in the same basal medium without any phytohormones and they subsequently grew into clumps of normal shoots. Rooting occurred with in vitro culture and during ex vitro acclimatization of T. fasciculata, however, the goal of this experiment was to form clumps of shoots for subculturing and multiplication.

3.5.1. Micropropagation of wild type T. fasciculata var. fasciculata

Seed explants of T. fasciculata var. fasciculata produced spherical clumps of shoots in half-strength MS media with equimolar of BA and IBA between the

range 0.3 and 10.0 mM. In terms of the number of shoots produced and the time required for multiplication, the multiplication media presented in this report compared favorably with published media for the micropropagation of bromeliads in the subfamily Tillandsioideae by seeds. A half-strength MS medium with equimolar BA and IBA at 1 and 10 mM produced more than 20 shoots per seedling in 4 months. A medium with equimolar 0.3 mM BA and IBA also proved to be excellent for the multiplication of T. ¯abellata seedlings (data not reported). Mercer and Kerbauy (1992) achieved a multiplication rate of about 22 shoots per seedling for Vriesia fosteriana after 3 months of culture in a solid

Knudson medium with 2.7 mM NAA and 8.9mM BA. Rogers (1984)micropropagated several Tillandsia species including T. dyeriana through seeds
by using different stage 1 and 2 media. The stage 1 medium contained coconut water and the stage 2 medium was solid vacin and went medium with 0.1 ppm NAA and 2 ppm BA. However, the rate of multiplication was not reported.

ANANAS COMOSUS L. SRIPAORAYA, SUNEERAT, MARCHANT, ROBERT, BRIAN POWER, J., DAVEY, MICHAEL R.. In Vitro Cellular and Developmental Biology - Plant **Volume:** 39 **Issue:** 5 **Pages:** 450-454

ABSTRACT

Axillary and terminal buds from suckers of *Ananas comosus* cv. Phuket were established on Murashige and Tucker-based (MT) medium with 2.0 mg l^{-1} (9.8 µ*M*) indolebutyric acid, 2.0 mg l^{-1} (10.74 µ*M*) naphthaleneacetic acid, and 2.0 mg l^{-1} (9.29 µ*M*) kinetin, followed by multiplication on Murashige and Skoog-based (MS) medium containing 2.0 mg l^{-1} (8.87 µ*M*) benzyladenine (BA) to provide a continuous supply of axenic shoots. Leaves, excised from such cultured shoots, produced adventitious shoots from their bases when these explants were cultured on MS medium containing 0.5 mg l^{-1} (2.26 µ*M*) 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.0 mg l^{-1} (8.87 µ*M*) BA. Embryogenic callus was produced when leaf explants were cultured on MS medium with 3.0 mg l^{-1} (12.42 µ*M*) 4-amino-3,5,6-trichloropicolinic acid (picloram). Somatic embryos developed into shoots following transfer of embryogenic tissues to MS medium with 1.0 mg l^{-1} (4.44 µ*M*) BA. Cell suspensions, initiated by transfer of embryogenic callus to liquid MS medium with 1.0 mg l^{-1} (4.14 µ*M*) picloram or 1.0 mg l^{-1} (4.52 µ*M*) 2,4-D, also regenerated shoots by somatic embryogenesis, on transfer of cells to semisolid MS medium with 1.0 mg l^{-1} (4.44 µ*M*) BA. All regenerated shoots rooted on growth regulator-free MS medium, prior to *ex vitro* acclimation and transfer to the glasshouse. These studies provide a baseline for propagation, conservation, and genetic manipulation of elite pineapple germplasms.

VRIESEA

Rech Filho, A. [Author]; Dal Vesco, L. L. [Author]; Nodari, R. O. [Author]; Lischka, R. W. [Author]; Mueller, C. V. [Author]; Guerra, M. P. [Author, Reprint Author; E-mail: mpguerra@cca.ufsc.br].

Institution	Univ Fed Santa Catarina, Lab Plant Dev Physiol and Genet, Grad Program Plant Genet Resources, BR-88034001 Florianopolis, SC, Brazil.
Title	Tissue culture for the conservation and mass propagation of Vriesea reitzii Leme and Costa, a bromeliad threatened of extinction from the Brazilian Atlantic Forest

Source Biodiversity & Conservation. 14(8). JUL 2005. 1799-1808.

Abstract The Brazilian Atlantic Forest biome is considered a hotspot of biodiversity. It is estimated that today the remaining primary vegetation covers only 7.5% of its original area. Bromeliad species are important components of this biome. Some of these species are endemic, like the highly endangered Vriesea reitzii. Tissue culture techniques have been often employed for the mass propagation and conservation of threatened bromeliad species. In the present work we describe a procedure for the micropropagation and in vitro conservation of V. reitzii. Seedling explants were cultured on MS and LPm liquid media supplemented with BA, NAA and GA(3). The induction and multiplication of shoots were observed in the MS medium supplemented with NAA (2 mu M) and BA (4 mu M). The best conditions for maintenance and conservation of shoots were half-strength or MS medium. Shoot elongation was observed in MS medium supplemented with GA(3) (10 mu M). MS medium supplemented with NAA (1 mu M) and BA (2 mu M) enabled an efficient proliferation system. The acclimatization of shoots longer than 2 cm resulted in 100% survival rate.

ISHS Acta Horticulturae 131: In Vitro Culture, XXI IHC

IN VITRO PROPAGATION OF *VRIESEA* CULTIVARS IN COMPARISON WITH OTHER ORNAMENTAL *BROMELIACEAE*

Authors O. Mekers, J.G. Van Onsem
:

Abstract:
For *Vriesea* cultivars we worked out a method of micro-propagation which in many respects is very different from the known methods for *Ananas*, *Aechmea* and *Cryptanthus*. This contribution gives a survey of the most important differences in the procedures. Before initiating a sterile culture and in order to obtain actively growing lateral buds in aseptic condition, the mother plants need a more drastical preparation period. To get better results in the sterilization of tissue explants, treatment with dikegulac after flowering and about two months before excising the buds, followed by a dry and clean period of at least two weeks are beneficial. The first stage of in vitro culture proves to be the most difficult and requires relatively much care. After 10 weeks, the resulting growing buds are transplanted on a multiplication medium. To obtain a mass of proliferating shoots, this second stage can be repeated a few times although normally it has to be alternated with a growth

period on an auxin containing medium. The third stage intends further elongation of the axillary shoots and rooting of the plantlets. Each stage takes about three months before transplanting on fresh medium is necessary. When the plants have reached 4 cm height, they are ready for greenhouse culture. If the last stage was hold on sufficiently, growth starts quickly with almost no adaptation problems.

ISHS Acta Horticulturae 78: Symposium on Tissue Culture for Horticultural Purposes

IN VITRO PROPAGATION OF SOME TILLANDSIOIDEAE (BROMELIACEAE)

Author: O. Mekers

Abstract:
Very little is known on the in vitro propagation of plants from the subfamily of the Tillandsioideae. The purpose of this work was to investigate their nutritional and physiological requirements.

Starting from sterilized seeds, it was possible to define optimal conditions for germination, growth, multiplication of plantlets and re-establishment to soil culture. The following factors were considered: agar solidified or liquid media, inorganic salt composition of Knudson versus Murashige and Skoog prescriptions; sugar concentration; growth substances (auxins, cytokinins and gibberellic acid).

Good propagation results were obtained for seedlings of Guzmania lingulata var. minor, Tillandsia polystachya, Vriesea heliconioides, Vriesca splendens and Vricsea 'Meyers' Favoriet'.

In order to prevent continuing axillary shoot proliferation special attention was paid to the pretransplant stage. Therefore, a supplementary culture on an auxin containing medium was necessary. After the first year in soil culture of a batch of several hundreds of plants of Vriesea 'Meyers Favoriet', originally propagated in vitro, no mutations in growth habit were observed.

ISHS Acta Horticulturae 78: Symposium on Tissue Culture for Horticultural Purposes

IN VITRO PROPAGATION OF SOME

ISHS Acta Horticulturae 78: Symposium on Tissue Culture for Horticultural Purposes

IN VITRO PROPAGATION OF SOME TILLANDSIOIDEAE (BROMELIACEAE)

Author: O. Mekers

Abstract:
Very little is known on the in vitro propagation of plants from the subfamily of the Tillandsioideae. The purpose of this work was to investigate their nutritional and physiological requirements.

Starting from sterilized seeds, it was possible to define optimal conditions for germination, growth, multiplication of plantlets and re-establishment to soil culture. The following factors were considered: agar solidified or liquid media, inorganic salt composition of Knudson versus Murashige and Skoog prescriptions; sugar concentration; growth substances (auxins, cytokinins and gibberellic acid).

Good propagation results were obtained for seedlings of Guzmania lingulata var. minor, Tillandsia polystachya, Vriesea heliconioides, Vriesca splendens and Vricsea 'Meyers' Favoriet'.

In order to prevent continuing axillary shoot proliferation special attention was paid to the pretransplant stage. Therefore, a supplementary culture on an auxin containing medium was necessary. After the first year in soil culture of a batch of several hundreds of plants of Vriesea 'Meyers Favoriet', originally propagated in vitro, no mutations in growth habit were observed.

(BROMELIACEAE)

Author: O. Mekers

Abstract:
Very little is known on the in vitro propagation of plants from the subfamily of the Tillandsioideae. The purpose of this work was to investigate their nutritional and physiological requirements.

Starting from sterilized seeds, it was possible to define optimal conditions for germination, growth, multiplication of plantlets and re-establishment to soil culture. The following factors were considered: agar solidified or liquid media, inorganic salt composition of Knudson versus Murashige and Skoog prescriptions; sugar concentration; growth substances (auxins, cytokinins and gibberellic acid).

Good propagation results were obtained for seedlings of Guzmania lingulata var. minor, Tillandsia polystachya, Vriesea heliconioides, Vriesca splendens and Vricsea 'Meyers' Favoriet'.

In order to prevent continuing axillary shoot proliferation special attention was paid to the pretransplant stage. Therefore, a supplementary culture on an auxin containing medium was necessary. After the first year in soil culture of a batch of several hundreds of plants of Vriesea 'Meyers Favoriet', originally propagated in vitro, no mutations in growth habit were observed.

Acta Horticulturae 572: XX International Eucarpia Symposium, Section Ornamentals, Strategies for New Ornamentals - Part II

CONTROLLING PREFERTILIZATION BARRIERS BY IN VITRO POLLINATION AND FERTILIZATION OF BROMELIACEAE

Authors: I. Vervaeke, E. Parton, R. Deroose, M.P. De Proft

Keywords: Bromeliaceae, cut style pollination, placental pollination, pollen-tube growth, *semi in vivo* pollination

Abstract:
Prefertilization barriers between different Bromeliaceae are mostly confined within the style. To control these barriers cut style, placental and placental grafted style pollination were studied. After cut style pollination the fertilization rate increased when a longer style part was left on the ovary of *Vriesea C*. Possibly less factors that control pollen tube guidance to the ovules are present in the lower style part. After placental pollination pollen germinated on the ovules but only rarely penetration of the micropyle by a pollen tube occurred. No difference in fertilization rate between compatible and incongruent pollination was found. *Semi in vivo* pollination resulted in a higher pollen tube growth out of the isolated style in comparison to *semi in vitro* pollination. However for *Vriesea C*, even after *semi in vivo* pollination pollen tube growth was inhibited at the stylar end. Grafting a style containing actively growing pollen tubes to the ovules on the placenta (placental grafted style pollination) resulted in a fertilization percentage, which was comparable to this after a classical pollination on the stigma for *Aechmea A*. Pollen tube growth through the style seems to be necessary for guiding pollen tubes to the ovule micropyle.